*Proc. Fla. State Hort. Soc*. 114:73-77. 2001.

**A PEER REVIEWED PAPER**

# A COMPARISON OF FIVE SOUR ORANGE ROOTSTOCKS AND THEIR RESPONSE TO CITRUS TRISTEZA VIRUS

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*Additional index words. Citrus aurantium*, vigor, flowering.

*Abstract.* **Sour orange (*Citrus aurantium* L.) clones were examined as potential parents for new hybrids that would possess good rootstock traits and tolerance to citrus tristeza virus (CTV). Five morphologically distinct clones were selected for detailed study. The clones varied in seedling vigor, with Sour No. 2 producing significantly more shoot and root biomass than Abers, Bittersweet, or Daidai, and Chinotto producing significantly less. Trees of 'Valencia' sweet orange (*C. sinensis* [L.] Osbeck) on each of the sour orange rootstocks were tested in the field with and without infection by isolates of CTV that typically cause a severe decline reaction in trees on sour orange rootstocks. In the absence of CTV infection, field vigor of grafted trees varied less than vigor of seedlings. After 20 months, uninfected trees on Sour No. 2 had significantly more scion biomass than trees on Bittersweet or Chinotto, but none of these were significantly different from trees on Abers and Daidai rootstocks. Trees on all the sour orange clones had severely reduced growth when infected with either T66 or T67 isolates of CTV for either 12 or 20 months. Trees on Sour No. 2 and Abers rootstocks had a significantly greater reduction of growth in response to infection by T67 than to infection by T66.**

Standard sour orange has many good characteristics as a rootstock for citrus, including resistance to blight, adaptability to a wide variety of soil conditions, and favorable influence on scion cold tolerance and fruit quality (Castle et al., 1993). However, trees grafted on sour orange rootstock are highly susceptible to some isolates of citrus tristeza virus (CTV) found in Florida (Brlansky et al., 1986). Mature field trees of sweet orange or grapefruit on sour orange rootstocks normally decline rapidly and die after they are infected with these common severe isolates of CTV. When trees on sour orange rootstocks in field trials are infected at an early age, they remain small and unproductive or die (Wutscher and Bowman, 1999).

Citrus tristeza virus has been responsible for losses of millions of trees with sour orange rootstock in Argentina, Brazil, and other countries (Lee and Bar-Joseph, 2000; Wallace, 1978). Estimates of tree losses to CTV in Florida and California vary, but a combination of CTV damage and CTV eradication efforts have destroyed millions of trees in each state. Historically, sour orange was used as a rootstock for about 30% of the citrus trees in Florida, but susceptibility to CTV has been responsible for a decrease in its use since the mid 1980s, to <0.5% of all trees planted in 2000 (Florida Department of Agriculture, 2000). Many Florida growers would like to find a new rootstock that combines the good traits of sour orange with resistance to CTV.

One viable approach to development of good new rootstocks is to create hybrids between sour orange and other CTV-resistant species. It is likely that the decline reaction to CTV will segregate in first or second generation hybrid progeny, and it should be possible to find selected hybrids bearing the good traits of sour orange but without a decline response to CTV. There are several important issues to address in the process of developing sour orange hybrids with good rootstock traits, including the selection of a sour orange clone as parent and the definition of efficient methods to identify hybrid rootstocks that will not have a decline or stunting reaction to CTV.

Many clones of sour orange are reported in the literature (Hodgson, 1967; Swingle, 1967), and all are normally identified as the species *C. aurantium.* However, sour orange is not a botanical species, but probably a group of closely related selections that originated through a natural interspecific hybridization. Molecular and morphological evidence suggest that the majority of the sour orange genome is derived from *C. grandis* (L.) Osbeck and *C. reticulata* Blanco (Barrett and Rhodes, 1976; Nicolosi et al., 2000). Most or all of the sour orange clones that are now found in germplasm collections were probably derived from one ancestral hybrid by a series of simple mutations, over many centuries, in vegetative tissue or nucellar embryos. Although all sour oranges are generally considered to be susceptible to some isolates of CTV when used as a rootstock for sweet orange, it is not clear whether they vary in the severity, rapidity, or isolate specificity of the reaction. There is little literature relating to rapid field testing of rootstock selections for resistance to CTV and the decline reaction. The short-term effect on growth of specific "decline–type" CTV isolates has also not been clearly defined as an assay for resistant or susceptible genotypes.

Objectives of these studies were to: 1) characterize variation in reaction to CTV among sour orange selections grafted with a sweet orange scion, 2) identify those with the best potential as parents in crosses, and 3) define conditions under which trees on sour orange rootstocks exhibit a measurable decline or stunting response to CTV so segregation among hybrids could be reasonably evaluated.

## Materials and Methods

Five morphologically distinct clones were selected from among the numerous sour oranges found in the USDA germplasm collection maintained at the A. H. Whitmore Foundation Farm, near Leesburg, Florida. Fruit and seeds were collected at that location from a single source tree (about 10 years old) of each sour orange clone. Source trees of Sour No. 2, Abers, Bittersweet, and Chinotto sour oranges were grafted on Swingle rootstock. The source tree of Daidai sour orange was grafted on Cleopatra rootstock.

*Fruit and seed traits.* Studies began in 1997 and continued as follows: Fruit length, diameter, and rind thickness were measured on 40 mature fruit of each clone in 1997, 1998, and 1999. Fruit weight, total acids, and total soluble solids were measured on four groups of 10 mature fruit of each clone in 1997, 1998, and 1999.

Mention of a trademark, warranty, proprietary product, or vendor does not imply an approval to the exclusion of other products or vendors that also may be suitable. Rootstock development by the USDA is supported in part by the Florida Citrus Production Research Advisory Council [Project 981-30I] and the Florida Citrus Research Foundation.

Number of seeds per fruit was measured on 10 fruit of each clone in 1999. Seed length, diameter, and weight were measured on 50 seeds of each clone in 1999.

*Seedlings grown in sand.* Seeds of each clone were planted during October 1998 in 3.7-L containers using steam-sterilized fine sand. Six pots of each clone were maintained in a warm greenhouse and watered as needed, alternating between non-amended well water and a water-soluble fertilizer mix, 15N-7P-14K (Peters Fertilizer Products, W. R. Grace, Fogelsville, Pa.) applied with a proportioner at a N rate of 380 $mg \cdot L^{-1}$. No supplemental light was supplied. The natural photoperiod fluctuated from 13.5 h in summer (using sunrise to sunset) to 10.25 h in December. Maximum photosynthetic photon flux (PPF) in the greenhouse was 800 $\mu mol \cdot s^{-1} \cdot m^{-2}$. After 4 months growth, plants were harvested and measured. The diameter of the shoot and root was taken at 3 cm above and below the cotyledon attachment point, respectively.

*Field testing with sweet orange scion.* Seeds were planted in a steam-sterilized peat/perlite/vermiculite potting mix (Pro-Mix BX: Premier Horticulture Inc., Red Hill, Pa.) at a rate of one seed per cell in multi-cell trays. After 3-4 months, selected true to type seedlings of each clone were transplanted into 3.7-L containers using the same potting mix formulation. At the time of transplanting, 0.5 g Kocide 101 (cupric hydroxide; Griffin Corp., Valdosta, Ga.) and 1 g Sequestrene 138 Fe (chelated iron; Ciba-Geigy Corp., Greensboro, N.C.) were applied to the containers to provide copper and iron to the growing plants. No supplemental light was supplied. Plants were maintained in a warm greenhouse and irrigated as described above for the seedlings in sand culture.

About 3 months after transplanting, seedlings were budded with CTV-free 'Valencia' sweet orange (clone 1-14-19) and selectively pruned to form a healthy sweet orange scion on the sour orange rootstock. Trees of each graft combination were divided into three groups: Two groups were inoculated with buds from a greenhouse source tree infected with one of two different CTV isolates and the third group was not inoculated. The T66 and T67 CTV isolates were used. T66 was originally isolated from a naturally infected 'Marsh' grapefruit tree on sour orange with strong decline symptoms at Ft. Pierce. T67 was obtained from severely stunted nursery trees of 'Hamlin' orange on sour orange at Avon Park. Both isolates previously induced stunting in grafted sweet/sour orange combinations and seedling yellows in sour orange seedlings under glasshouse conditions.

Two blind buds from the inoculum source trees were inserted into each scion in the infected treatments about 8 cm above the graft union. Trees were monitored for 4 weeks and rebudded as necessary to ensure two live source buds in each inoculated test tree. Trees on the five sour orange rootstocks and with the three CTV treatments were planted at 1.5m by 0.5m spacing into a field plot near Plymouth, Fla. (Orange County) in July 1998 using a randomized design. At least seven trees were included for each treatment. Trees were fertilized with dry fertilizer at recommended rates and irrigated three times per week by overhead sprinkler. Scion and rootstock calipers were measured on test trees at planting, and after 6, 12, and 20 months. Flowering of trees was scored in April (about 10 months after planting) using a 0-3 scale, with 0 = no flowering and 3 = tree heavily covered with flowers. All initial fruit set from blossoms was removed promptly. Scion biomass (fresh weight) was measured when trees were harvested at 20 months after planting. Leaf samples were collected from trees in March 1999 (about 9 months after planting) and tested for presence of the T66 and T67 CTV using MCA13 Enzyme-Linked Immunosorbent Assay (ELISA) (Nikolaeva et al., 1998; Permar et al., 1990). Trees that were identified to have the wrong infection status (ie., MCA13 negative for a T67 inoculated tree, or vice versa) were eliminated from the analysis.

The data were tested by analysis of variance using Statistica ver 5.0 (StatSoft, Tulsa, Okla.) and comparison of the means was across rows by Duncan's multiple range test at $P<0.05$. Percent reduction of value by T67 infection versus uninfected control was calculated for scion and rootstock calipers, scion biomass, and flowering. In addition, significance of the T67 effect was determined by t-test.

## Results

The sour orange clones Abers, Chinotto, and Daidai could be readily discriminated from each other and the other two clones examined in this study by casual visual observation of at least one gross morphological feature that distinguishes each. In general, these traits also differentiate the three cultivars from most other common sour orange clones. Abers has a very narrow leaf blade, Daidai has fruit with a fleshy persistent calyx, and Chinotto has many dwarf features, such as small leaves, fruit, and short internodes. Quantitative comparison of fruit and seed traits identified numerous other significant differences among the five clones (Table 1).

Table 1. Fruit and seed characteristics of sour orange clones[z]

| | Sour No. 2 | Abers | Bittersweet | Daidai | Chinotto |
|---|---|---|---|---|---|
| Fruit length (mm) | 68 b | 68 b | 68 b | 80 a | 44 c |
| Fruit diameter (mm) | 78 b | 68 c | 79 b | 95 a | 55 d |
| Rind thickness (mm) | 8 b | 7 c | 8 b | 11 a | 4 d |
| Individual fruit weight (g) | 207 b | 156 c | 207 b | 324 a | 74 d |
| Total acid (%) | 5.1 a | 4.8 b | 3.9 c | 1.7 d | 0.6 e |
| Total soluble solids (%) | 10.5 a | 10.2 ab | 9.9 b | 9.2 c | 10.1 b |
| Number of seeds per fruit | 32 ab | 25 bc | 19 cd | 38 a | 16 d |
| Seed weight (mg) | 163 b | 158 b | 160 b | 208 a | 161 b |
| Seed length (mm) | 14.2 a | 13.5 b | 13.7 b | 14.4 a | 12.2 c |
| Seed diameter (mm) | 6.4 c | 6.5 bc | 6.4 c | 7.2 a | 6.7 b |

[z]Mean separation across rows by Duncan's multiple range test at $P < 0.05$.

Vigor of seedlings grown in sand varied considerably among the five clones (Table 2). Shoot length of Sour No. 2 was more than

double that of Chinotto, and total seedling fresh weight (shoot plus root) was more than five times greater. Chinotto was significantly smaller than Sour No. 2 in every measure of relative tree seedling size. Abers, Bittersweet, and Daidai formed an intermediate group that was significantly different from either Sour No. 2 or Chinotto for many of the measurements.

In grafted trees with 'Valencia' scion, mean scion caliper of uninfected trees did not differ significantly between rootstock clones at field planting and ranged from 6.5 to 7.4 mm (analysis not shown). Field vigor of grafted trees on the different clones also varied much less than vigor of seedlings. After 20 months, uninfected trees on Daidai, Abers, Chinotto, and Bittersweet were not significantly different from each other in final biomass, or scion or rootstock calipers (analysis not shown). Final mean scion calipers for the uninfected treatment ranged from 32.26 mm for trees on Bittersweet (the smallest) to 36.61 mm for Sour No. 2. Uninfected trees on Sour #2 were significantly larger than trees on Bittersweet or Chinotto as measured by final scion biomass, but not distinguishable from any of the clones in final scion or rootstock caliper. Much of the difference in vigor of seedlings was not evident when the sour orange shoot was replaced by a common sweet orange scion.

ELISA testing of the field trees after 9 months identified three trees in the uninoculated treatments with a very high reaction to MCA13, probably resulting from natural aphid transmission of CTV to these trees in the field. Three trees in the inoculated treatments were also identified with very low reaction to MCA13 and were probably due to a failure of the CTV inoculation of these individual trees. All six trees determined by ELISA to be of the incorrect infection status were eliminated from comparisons of the CTV treatments at all time periods.

During the first 6 months that the trees were in the field, infection of trees with T66 or T67 isolates did not significantly reduce tree growth on any of the five sour orange rootstock clones, as measured by scion and rootstock calipers. Mean scion caliper increases for uninfected trees during this period were 8.87 mm for Sour No. 2, 7.82 mm for Abers, 7.39 mm for Bittersweet, 8.47 mm for Daidai, and 8.05 mm for Chinotto.

In contrast, there was a highly significant reduction of caliper growth on Sour No. 2, Abers, Bittersweet, and Daidai by either T66 or T67 infection during the period of 6-12 months after field planting (Table 3). During this period, growth of trees on Chinotto was reduced by infection with T66 or T67, but to a less significant degree. There was no evidence of a difference between the effect of the two isolates on caliper growth through 12 months of age for any of the sour orange clones.

From 12-20 months after field planting, all the sour orange clones exhibited highly significant reduction of caliper growth by infection with either T66 or T67 isolates (Table 4). For the clones Sour No. 2 and Abers, reduction of caliper growth by T66 infection was significant, but less than reduction by infection with T67.

Trees on all five sour orange clones had a highly significant reduction of scion biomass from CTV infection after 20 months in the field (Table 5). Examined separately, none of the rootstock clones could be shown to have a significantly different biomass reaction to T66 than to T67. However, for Sour No. 2, Abers, and Daidai, the trend appears to be a more severe reduction in tree growth by T67. When data for all five sour oranges was pooled, there was a significantly greater reduction of tree growth by T67 than T66. Flowering of trees on Sour No. 2, Abers, and Bittersweet was significantly increased by CTV infection. There was no evidence for any significant effect of CTV on flowering of trees on Daidai or Chinotto.

## Discussion

The relatively large variation in morphological features that was documented among the five sour orange clones might be representative of as much genetic diversity as is available within the common selections of true sour oranges (although this is known to be small compared with differences between sour orange and other citrus species). It was hoped, but could not be proven, that this would provide a range in growth and CTV responses representative of those that might be commonly found among sour oranges. Most of these traits probably are unimportant in assessing the relative value of each sour orange clone as a rootstock. Seediness is a trait that is important for commercial propagation of rootstocks, but all the clones (including the least seedy, Chinotto) probably produced enough seed for economic commercial use.

There was a surprisingly large variation in seedling vigor between the five sour orange selections. Vigor is probably important in assessing relatively ease of commercial nursery propagation with each clone as a rootstock. In particular, the exceptionally slow growth and short internode length of Chinotto seedlings would make this clone difficult to use in commercial propagation. However, it appeared that grafted trees with a 'Valencia' scion in the field varied little in growth rate, and seedling vigor might not be of much importance in actual field performance of grafted trees. It was not clear why use of a sweet orange scion reduced the variation

Table 2. Seedling characteristics of sour orange selections after 4 months[z]

| | Sour No. 2 | Abers | Bittersweet | Daidai | Chinotto |
|---|---|---|---|---|---|
| Shoot length (mm) | 295 a | 267 a | 208 b | 196 b | 137 c |
| Shoot diameter (mm) | 4.0 a | 3.9 a | 3.9 a | 3.7 a | 2.2 b |
| Shoot fresh weight (mg) | 8935 a | 7319 b | 5476 c | 5582 c | 1664 d |
| Shoot dry weight (mg) | 3106 a | 2530 b | 1826 c | 2011 c | 488 d |
| Number of leaves | 20 b | 19 bc | 17 bc | 16 c | 31 a |
| Total leaf area ($cm^2$) | 299.9 a | 233.4 b | 194.4 b | 183.5 b | 57.2 c |
| Single expanded leaf area ($cm^2$) | 25.9 a | 25.4 a | 20.8 b | 21.6 ab | 3.2 c |
| Root fresh weight (mg) | 6267 a | 4206 b | 3703 b | 3979 b | 1321 c |
| Number root branches | 451 a | 389 ab | 332 b | 356 ab | 179 c |
| Root diameter (mm) | 4.1 a | 4.1 a | 3.9 a | 3.9 a | 2.2 b |

[z]Mean separation across rows by Duncan's multiple range test at $P < 0.05$.

Table 3. Scion and rootstock caliper increase (mm) of 'Valencia' on sour orange rootstocks between 6-12 months age.[z]

| Clone | Uninfected | T66 | T67 | T67 effect (%)[y] |
|---|---|---|---|---|
| Sour No. 2 | | | | |
| scion | 5.33 a | 2.37 b | 2.18 b | -59*** |
| rootstock | 7.45 a | 3.63 b | 3.49 b | -53*** |
| Abers | | | | |
| scion | 4.98.a | 2.14 b | 2.48 b | -50*** |
| rootstock | 7.01 a | 3.68 b | 3.52 b | -50*** |
| Bittersweet | | | | |
| scion | 4.95 a | [x] | 2.24 b | -55*** |
| rootstock | 6.84 a | [x] | 3.48 b | -49*** |
| Daidai | | | | |
| scion | 4.88 a | 2.83 b | 2.01 b | -59*** |
| rootstock | 7.91 a | 3.95 b | 3.05 b | -61*** |
| Chinotto | | | | |
| scion | 5.12 a | 3.28 b | 3.57 b | -30* |
| rootstock | 6.51 a | 3.68 b | 5.00 ab | -23 |
| Average | | | | |
| scion | 5.04 a | 2.67 b | 2.49 b | -51*** |
| rootstock | 7.12 a | 3.74 b | 3.70 b | -48*** |

[z]Values shown as caliper increase (mm) between 6 months and 12 months (tree growth), and effect of T67 infection (percent). Scion and rootstock values are compared across rows, respectively. Mean separation across rows by Duncan's multiple range test at $P < 0.05$.
[y]Percent reduction of uninfected value by T67 infection. Significance of T67 effect determined by t-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
[x]A treatment of sweet orange on Bittersweet rootstock with T66 isolate was not tested.

Table 4. Scion and rootstock caliper increase (mm) of 'Valencia' on sour orange rootstocks between 12-20 months age.[z]

| Clone | Uninfected | T66 | T67 | T67 effect (%)[y] |
|---|---|---|---|---|
| Sour No. 2 | | | | |
| scion | 15.23 a | 7.61 b | 3.92 c | -74*** |
| rootstock | 19.97 a | 10.53 b | 6.57 b | -67*** |
| Abers | | | | |
| scion | 14.63 a | 7.80 b | 4.76 c | -67*** |
| rootstock | 21.47 a | 11.15 b | 7.47 c | -65*** |
| Bittersweet | | | | |
| scion | 13.37 a | [x] | 4.59 b | -66*** |
| rootstock | 17.31 a | [x] | 7.86 b | -55*** |
| Daidai | | | | |
| scion | 12.98 a | 8.29 b | 6.05 b | -53*** |
| rootstock | 16.87 a | 11.62 b | 8.88 b | -47*** |
| Chinotto | | | | |
| scion | 13.13 a | 7.35 b | 5.39 b | -59*** |
| rootstock | 17.78 a | 10.17 b | 11.40 b | -36** |
| Average | | | | |
| scion | 13.86 a | 7.77 b | 5.00 c | -64*** |
| rootstock | 18.61 a | 10.88 b | 8.52 c | -54*** |

[z]Values shown as caliper increase (mm) between 12 months and 20 months (tree growth), and effect of T67 infection (percent). Scion and rootstock values are compared across rows, respectively. Mean separation across rows by Duncan's multiple range test at $P < 0.05$.
[y]Percent reduction of uninfected value by T67 infection. Significance of T67 effect determined by t-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
[x]A treatment of sweet orange on Bittersweet rootstock with T66 isolate was not tested.

Table 5. Flowering at 10 months and scion biomass at 20 months for 'Valencia' on sour orange rootstocks.[z]

| Clone | Uninfected | T66 | T67 | T67 effect (%)[y] |
|---|---|---|---|---|
| Sour No. 2 | | | | |
| flowering | 0.22 b | 2.0 a | 2.86 a | +1200*** |
| biomass | 3697 a | 1138 b | 646 b | -83*** |
| Abers | | | | |
| flowering | 0.22 b | 1.78 a | 2.14 a | +873*** |
| biomass | 3176 a | 1306 b | 595 b | -81*** |
| Bittersweet | | | | |
| flowering | 0.21 b | [x] | 1.88 a | +795*** |
| biomass | 2357 a | [x] | 668 b | -72*** |
| Daidai | | | | |
| flowering | 1.00 | 2.00 | 2.22 | +122 |
| biomass | 3241 a | 1348 b | 795 b | -75*** |
| Chinotto | | | | |
| flowering | 0.00 | 0.00 | 0.12 | [w] |
| biomass | 2474 a | 939 b | 976 b | -61*** |
| Average | | | | |
| flowering | 0.32 b | 1.43 a | 1.82 a | +469*** |
| biomass | 2938 a | 1184 b | 743 c | -75*** |

[z]Values shown as flowering at 10 months (0-3 score), scion biomass (g) at 20 months, and effect of T67 infection. Flowering and biomass values are compared across rows, respectively. Mean separation across rows by Duncan's multiple range test at $P < 0.05$.
[y]Percent change from uninfected value by T67 infection. Significance of T67 effect determined by t-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
[x]A treatment of sweet orange on Bittersweet rootstock with T66 isolate was not tested.
[w]Percent effect was not calculable and effect was not significant.

in tree growth rate among the sour orange clones. In the case of Chinotto, the very short internodes and small leaves formed by the seedlings may be inadequate to generate sufficient photosynthetic surface to support more vigorous growth (Table 2).

Conspicuous, abundant flowering had been previously identified as an early symptom of CTV decline in young trees (Wallace, 1978). Although this effect was evident in three of the sour orange clones tested, it did not appear consistent across all sour orange clones.

The dramatic effect of T66 and T67 infection on caliper increase and biomass was consistent across all five sour orange clones. This effect was first observed 6-12 months post-inoculation, and continued or strengthened through 20 months. There did not appear to be any important differences between the sour orange clones in reaction to CTV that would favor use of one over the others in the creation of new sour orange hybrid rootstocks. There was some evidence for CTV isolate differences in severity of growth response for some sour orange clones. While this may be of some interest, it did appear that either T66 or T67 could be used to reveal a stunting reaction in susceptible sour orange rootstocks.

Under Central Florida field conditions, it appeared that a "sour orange-like" stunting reaction could be clearly observed within 12-20 months after field planting, but not earlier. Preliminary testing of sour orange hybrids for a stunting reaction to CTV could be completed with some confidence within the same amount of time. Conclusive proof of durable tolerance to severe CTV strains in the field would require confirmation through long-term field trials.

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*Proc. Fla. State Hort. Soc*. 114:77-79. 2001.

# SPECTRAL REFLECTANCE OF CITRUS CANKER

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*Abstract.* **Duncan grapefruit trees were inoculated with citrus canker. The spectral reflectance of individual leaves was measured and found to change as canker lesions develop. The spectral change was most pronounced in the 600-700 nm spectral region. Reference spectra were treated as vectors in a linear system, and the extent of lesion coverage was estimated. Performing a similar analysis with spectra from iron deficient, manganese deficient, and leaves with greasy spot symptoms showed the uniqueness of the spectrum of the citrus canker lesion and the potential for this technology for assessing the extent and magnitude of canker infection.**

Reflectance spectra of vegetation, measured in the 400 nm to 2500 nm region of the electromagnetic spectrum, contain information on plant pigment concentration, leaf cellular structure, and leaf moisture content. This allows the remote discrimination and mapping of plant species or communities, the detection of their physiological condition and state of health, and the assessment of the amount of cover or biomass, based on the unique spectral properties of a vegetated surface. High-spectral-resolution data sets in image format demonstrate the potential application of such data for a variety of environmental applications. Work by Blaquez (1990, 1991, 1992, 1993) has demonstrated the potential of this technology for agricultural applications.

Spectral reflectance measurements were taken with an ASD (Analytical Spectral Devices, Boulder, Colo.) FieldSpec FR spectroradiometer with a spectral range of 350-2500 nm. The spectral measurements were taken indoors and a Lowel Pro-lamp interior light assembly provided illumination. The spectral reflectance for each sample was measured four times; each subsequent measurement was at a slightly different orientation than the previous measurement to account for any bi-directional effects. No bi-directional reflectance effect or spectral variability as a function of illumination or viewing geometry was observed for any of the samples. The four spectral reflectance measurements were averaged to produce a reference spectrum. Each reference spectrum was added to a spectral database, or spectral library.

## Materials and Methods

Duncan grapefruit (*Citrus paradisi* Macf.) seedlings were grown in 9.5 × 24 cm citrus pots for the study and maintained in a greenhouse at 30°C through the duration of the study. An inoculum suspension of *Xanthomonas anoxopodis pv. citri* was prepared by culturing on nutrient agar for 48 h and the colonies were washed from the media surface with phosphate buffer. The final concentration was adjusted to $1 \times 10^8$ and used to inoculate I expanded leaves via a pinprick method. After 30 d, ten lesions approximately 5 mm in diameter were excised from the leaves and ground in 20 ml of phosphate buffer. The suspension was used to inoculate additional leaves on healthy trees in the manner described above, however only one inoculation point was located per leaf.

Three sets of spectral reflectance measurements were taken from each of four trees. One measurement was taken on a leaf that was not inoculated, and the other two measurements were taken on an inoculated leaf. The measurements were taken immediately be-